1211-Pos Board B121

Dynamics Investigation of the Cytochrome P450cam Active Site Mutant Thr252ALA

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Cytochrome P450s are vital heme-containing enzymes that facilitate catalysis in a wide variety of biochemical processes including carcinogenesis, drug metabolism, steroid biosynthesis and toxin degradation. Because of its ability to catalyze the hydroxylation mechanism of camphor in its active site, Cytochrome P450cam served as the model system for studying P450s. The crystal structure of a P450cam mutant, in which the active site Threonine252 (Thr252) has been replaced with an alanine (ala) residue (Thr252Ala), was solved at 2.2Å resolution. According to sequence alignment analysis, Thr252 is highly conserved among P450s, and it was suggested to be an essential active site residue, forming part of the dioxygen-binding site. Mutation of the active site Thr to Ala changes local conformation of the active site residues and affects hydroxylation of the substrate, producing hydrogen peroxide and ''excess'' water instead of the substrate polarizing product, 5-exo-hydroxycamphor. In the mutant active site, a solvent not present in the native enzyme is positioned in the dioxygen-binding region and the network of hydrogen bonds is restructured accordingly. These factors are believed to be critical to enzymatic reaction changes of P450cam mutant from the wild-type, most notably the proton delivery. Molecular dynamics (MD) simulation has been performed on the mutant to predict the relevant structural dynamics, especially those of the active site residues. Neutron scattering (NS) spectra calculations have been extrapolated from the simulation and compared with the results with the indicated crystallographic findings and those of the wild-type P450cam simulations already empirically verified via experimental neutron scattering. Preliminary results predict that local interactions around the heme and cam site have been altered midway through the trajectory. The preliminary data has also provided insight to understanding the local water diffusion dynamics of the CYP450cam mutant active site.

1212-Pos Board B122

Making Substrates Out of Inhibitors: Distal Cavity Mutations in Dehaloperoxidase from Amphitrite Ornata

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Dehaloperoxidase (DHP A), a unique multi-function enzyme, from the marine worm *Amphitrite ornate* dehalogenates 2,4,6-trihalophenols to form the corresponding 2,6-dihalogenated quinone product. The catalytic cycle of DHP is similar to horseradish peroxidase (HRP), involving a high-valent oxoferryl heme and two one electron transfers from the aromatic substrate to the enzyme. However, unlike HRP, DHP has an internal binding cavity on the distal side of the heme capable of accommodating monohalogenated phenols. Internal binding of monohalogenated phenols in the distal cavity of DHP inhibits peroxidase function. Therefore, even though the peroxidase mechanism of DHP is similar to HRP, DHP is not capable of oxidizing the same range of halogenated substrates. Blocking internal binding in DHP may be the key to effectively enable DHP to function as a peroxidase on the full range of halogenated phenols. The distal cavity of DHP is surrounded by several hydrophobic amino acids that stabilize internal binding of the monohalogenated phenols: several phenylalanine residues (F21, F24, and F35), a valine residue (V59), and a leucine residue near the back edge of the heme (L100). We have recently expressed the L100F, L100Q, L100T, and L100N mutants of DHP in an effort to prevent internal binding and turn the inhibitors into substrates. These mutants are being characterized by UV-vis spectroscopy, resonance Raman spectroscopy, X-ray crystallography, and molecular dynamics simulations. Kinetic assays indicate that the peroxidase activity of the L100 mutants is reduced compared to native DHP, and although we believe it to be possible theoretically, none of the L100 mutations have caused the switch from inhibitor to substrate up to the present.

1213-Pos Board B123

Proposed Mechanism for Reductively Driven Hybrid b/c Heme Formation in Synechocystis and Synechococcus Hemoglobins

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The heme group is used for a variety of biological functions including electron transport, O_2 activation, ROS detoxification and reversible ligand binding. One way in which proteins alter heme reactivity is through covalent modification, as illustrated in the *c*-type cytochromes, which anchor the heme to the polypeptide through thioether bonds between the vinyl groups and cysteines. The chemical details of *c* heme generation in vitro, however, remain to be elucidated.

The cyanobacteria *Synechocystis* sp. PCC 6803 (S6803) and *Synechococcus* sp. PCC 7002 (S7002) each possess a single gene encoding a hemoglobin. In the test tube, these hemoglobins (GlbNs, 59% identity) undergo a post-translational modification by which His117, in the H helix, adds to the 2-vinyl Calpha to form a hybrid *b/c* heme. Interestingly, cross-linked S7002 GlbN is obtained from *Synechococcus* cells,[1] which confirms that the linkage is physiologically relevant.

Using NMR and rapid mixing optical absorbance spectroscopies, we gathered experimental evidence supporting an electrophilic addition mechanism for the post-translational modification in ferrous S6803 and S7002 GlbNs. Specifically, rate-determining labile H^+ addition occurs irreversibly at the 2-vinyl Cbeta generating an alpha carbenium ion. The carbocation then undergoes nucleophilic attack by His117, leading to the observed Markovnikov adduct. Reduced His117Cys S6803 GlbN is capable of forming a thioether bond analogous to that in *c*-typecytochromes. It is concluded that the mechanism has validity beyond the particular case of cyanobacterial hemoglobins and may apply to cytochrome c as well.

1. Scott NL *et al*. (2010) Biochemistry 49:7000-7011

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1214-Pos Board B124

Distal Histidine Flexibility as the Key to the Reactivity of Dehaloperoxidase-Hemoglobin

Matt Thompson, Stefan Franzen.

The enzymes dehaloperoxidase A and B (DHP A&B) from the marine worm Amphitrite ornata are unique hemoglobins that function as peroxidases, capable of converting 2,4,6-trihalo- phenols into the corresponding 2,6-dihalogenated quinones. It is difficult to explain the peroxidase activity of DHPs A&B since they are clearly both hemolgobins. Our recent discovery of an internal inhibitor binding site, in the distal pocket, clearly establishes the peroxidase function and suggests that the distal histidine (H55) plays the central role in both function switching and activation of DHPs A&B. Similar to many myoglobins, H55 of DHP A is observed in two conformations, open and closed. However, in DHP A, H55 has been shown to exist in the open conformation to a much greater extent than in any known myoglobin. This results in an open exit channel for bound diatomic ligands, which leads to rapid escape. However, the open conformation may also permit the entry of hydrogen peroxide and even the native inhibitor, 4-bromophenol. We also have structural and spectroscopic evidence that external binding of substrate pushes H55 more deeply into the protein. This may serve to aid in the activation of bound hydrogen peroxide as part of the peroxidase mechanism. Thus, in DHP A, the closed and open states are inactive and active, respectively, for peroxidase chemistry. We have systematically probed this hypothesis using a variety of methods and site-directed mutants to determine the role played by the distal cavity in the stabilization of the inhibitor and the flexibility of the distal histidine. Based on the binding of the series of 4-halophenols in X-ray crystal structures, it is evident that there is a protein cavity deep inside DHP A. The functional relevance of this cavity will be elucidated.

1215-Pos Board B125

Interplay Between Ligand and Substrate Binding in Human Indoleamine 2,3-Dioxygenase

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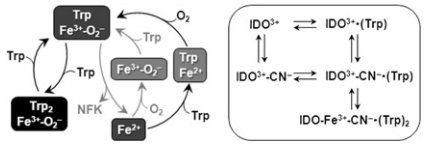
Human indoleamine 2,3-dioxygenase (hIDO) is a heme-containing enzyme that catalyzes the initial and rate-determining step of L-tryptophan (L-Trp) metabolism via the kynurenine pathway. Earlier kinetic studies showed that the K_m of L-Trp (0.015 mM) is ~27-fold lower than the Kd (0.4 mM) for the ligand-free ferrous enzyme, suggesting that O_2 binding proceeds L-Trp binding during the catalytic cycle. With cyanide as a structural probe, we have investigated the thermodynamic and kinetic parameters associated with ligand and substrate binding to hIDO. Kinetic studies demonstrate that pre-binding of L-Trp to the enzyme retards cyanide binding by ~13-fold, while pre-binding of cyanide to the enzyme facilitates L-Trp binding by ~22-fold. The data support the view that during the active turnover of the enzyme it is kinetically more favorable to bind O_2 prior to L-Trp. Equilibrium titration studies show that the cyanide adduct is capable of binding two L-Trp molecules, with K_d values of 0.018 and 26 mM. The data offer the first direct evidence of the second substrate binding site in hIDO, underlying its substrate-inhibition behavior.



1216-Pos Board B126

Using X-rays to Watch Proteins Function with 100 Picosecond Time Resolution

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To generate a deeper understanding into the relations between protein structure, dynamics, and function, we have developed time-resolved X-ray methods